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MEASUREMENT OF VIRUS ACTIVITY IN PLANTS

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One of the most important contributions to the study of plant viruses since the discovery of these infectious agents by Beijerinck (5) in 1898 was the observation by Holmes (11) in 1929 that tobacco mosaic virus would produce necrotic local lesions at the point of inoculation on certain species of plants. This observation paved the way for the discovery of local lesions produced by other viruses. It permitted the separation of virus strains from a mixture, much as the poured plate method of Koch provided a means of isolating pure cultures of bacteria, and thus led directly to the proof that viruses mutate. It made possible the development of methods for quantitative measurements of virus activity and therefore allowed accurate studies of a host of problems on this and other viruses, including biological and physical properties, purification and characterization, nature of infection, and the routes and rates of movement and multiplication within plants.

As knowledge concerning plant viruses has increased, the need for methods to measure small differences in virus activity has become greater. To understand the refinements in technique which have made such measurements possible it will be necessary to consider first of all the infectious unit and the nature of its activity-dilution curve.

The Virus Units

There is now abundant evidence that the plant viruses with which we are here concerned are spherical or rod-shaped bodies ranging in size from 10 mμ to 300 mμ in length and composed chiefly of nucleoprotein. They have not been grown in cell-free media but reproduce abundantly when introduced into the cells of an appropriate host. The most effective method of introducing them into a host is to rub a solution containing them across the leaf surface with sufficient pressure to cause many minute injuries to the epidermal cells. This is usually done with a cloth pad, previously moistened with the virus solution, but glass spatulas such as those designed by Samuel (18) are used in some instances. The leaf to be inoculated rests in the palm of one hand and pressure is applied with the other.

It is well established that for infection to take place the injury to the leaf must be made in the presence of the virus solution. Rubbing with a dry cloth followed by the application of virus without pressure does not cause infection. Whether injury to the protoplast of the cell is followed by a brief suction, which pulls virus into the cell, and then by rapid healing is not definitely known, but it is apparent that entrance of virus into the cell is practically simultaneous with injury to the cell. If the solution is washed from the leaf surface immediately after inoculation the number of lesions is not decreased; there is often an increase, presumably due to the removal of

toxic materials in the inoculum that might cause further injury or prevent healing of the previously injured cells.

With such a crude method of inoculation, it is apparent that the attendant variation in response of the host will indeed be great. An underlying cause of this variation is the variability in the number of cells injured in just the right manner as the virus is rubbed across the leaf surface. There are also many other variables and it is a problem in experimental design and in statistics to separate this variation from that due to differences in activity of the solutions being tested.

Nature of Infection

Two theories have been advanced to account for the sigmoidal shape of the curve obtained when the number of infections is plotted as a function of the logarithm of virus concentration. One of these, proposed by Bryan and Beard (7), is a modification of the theory used by Gaddum (8) to explain the response of organisms to varying dosages of drugs. It holds that when a virus solution is brought into contact with susceptible host tissue, defense mechanisms are marshalled for combating the invaders; infection results when the quantity of virus in the inoculum is sufficient to overcome the defense mechanisms. The theory postulates that host susceptibility varies in such a manner that the distribution of the logarithms of dose susceptibilities follows the normal law. When the standard deviation of the distribution of dose susceptibilities was taken as 0.5 log units, the theoretical curve coincided with the ones observed experimentally.

The second theory is based on the assumption that infection is determined by the chance occurrence of a minimum number of virus particles in unit volume of solution that comes into contact with a susceptible region of a host. Signs that infection has occurred result from the multiplication of these particles, not, as in the case of a drug, from the effect of the actual particles introduced. Consider the particles of a virus dispersed uniformly in a liquid medium. As successive serial dilutions of the solution are taken, the probability of finding a particular number of particles in a unit volume of the solution will be given by the terms of the Poisson series. The probability that at least one particle of virus will be found in v cc of a dilution x (eg. ½, 1/10) of a virus sample containing n particles per cc is $1-e^{-vnx}$ This is also the probability of obtaining infection if a single virus particle coming into contact with a susceptible region of a host is able to infect. If y be taken as the actual number of infections obtained and N as the theoretical maximum number, then the graph relating dosage and response will be given by

$$y/N=1-e^{-vnx} \qquad (1)$$

If 2 particles are required for infection, the equation becomes

$$y/N=1-e^{-vnx}-vnxe^{-vnx} \qquad (2)$$

These equations were developed for bacterial infections by Greenwood and Yule (9) and Halvorson and Ziegler (10) and for virus infections by Lauffer and Price (12).

Youden, Beale, and Guthrie (24) and Bald (1,2) independently showed that dilution curve data for several plant viruses could be fitted to the form of equation 1. Bryan and Beard (7), on the other hand, felt that a better fit for experimental dilution curve data was obtained by application of the theory of Gaddum (8). Recently, the problem was re-examined by Lauffer and Price (12). They found three reasons for regarding as untenable the assumption that infection depends upon the outcome of a battle between the defense mechanisms of the host and the virus solution in contact with the host (the Gaddum theory). The first of these reasons is that many of the available data fit the alternative hypothesis better. The second reason is that the dilution curves for all viruses so far studied tend to have the same shape, a fact which requires, if the Gaddum theory is to apply, that variability in host susceptibility is a universal constant. This is an unlikely possibility which is not required by the alternative hypothesis. The third reason is that the distribution of mixed infections in local lesions produced by various dilutions of an inoculum containing 2 distinguishable strains of virus was inconsistent with the Gaddum theory but not with the theory that only a single particle is required for infection. Lauffer and Price showed definitely that, if the chance occurrence theory is correct, then equation 1, rather than equation 2 or the equa-

tions involving more than 2 particles, is the only one that fits the data.

Half-Leaf Method of Measurement

It has been pointed out by Samuel and Bald (19) that the most accurate way of determining whether two virus preparations differ in activity is to test them on opposite halves of the same leaves. The solutions should be alternated between left and right halves to overcome the bias of the operator or the actual difference in susceptibility, if such exists. The significance of the difference between virus samples can be tested by applying Student's "t" test, or by analysis of variance (23).

Beale (4) using the half-leaf technique, found that differences in concentration of tobacco mosaic virus as small as 50 per cent could be detected, with odds of 50:1 or better, when from 9 to 16 *Nicotiana glutinosa L.* plants were inoculated. Loring (13) found that differences of 10 per cent or greater could be detected when from 40 to 50 half leaves of *Phaseolus vulgaris L.* were used. With *N. glutinosa* as the test plant, the smallest difference that was readily distinguished was 20 per cent. Loring pointed out that these findings were valid only when the virus protein concentrations used were in the vicinity of 10^{-6} gm./cc. Youden and Beale (23) suggested the use of a latin-square arrangement of test plant leaves for comparing activities of more than two preparations. Since the numbers of useful leaves on a plant are limited, Youden (25) adapted the method of incomplete blocks (Youden squares) for testing differences between virus samples when their number exceeds th number of leaves on a plant.

It is an essential feature of the graph of equation 1 that, when log y/N is plotted as a function of log vnx, a considerable region of the curve can be represented by a curve approximating a straight line with a slope of unity. Thus it should be possible to interpret numbers of infections produced by a virus sample of an appropriate dilution directly in terms of virus activity. However, not all virus dilution data follow equation 1 precisely; as a matter of fact most of the local lesion data for plant viruses, fail to do so. Bald (3) concluded that the distortion of the dilution series in such cases might be explained on the assumption that some virus samples are partly aggregated and that the aggregates dissociate into active particles on dilution. If b is taken as the number of aggregates then the equation becomes

$$y/N = 1 - e^{-vbx} \qquad (3)$$

and vbx may be solved in terms of vnx to give

$$vbx = \frac{-1 \pm \sqrt{1+4Kvnx}}{2K}$$

where K is a constant. Equation 3 describes a curve similar to equation 1 but with a flatter slope, depending upon the value of K. Moreover, since the degree of aggregation, and hence K, will vary from experiment to experiment the slope of the dilution curve cannot be predicted ahead of time with certainty. Consequently, if it is desired to translate differences in lesion counts into differences in virus concentration, or activity, it is necessary to design the experiment in such a manner that the slope of the dilution curve as well as the differences in lesion counts can be ascertained from the same data.

One method of doing this has been suggested by Loring (13). It is to test the unknown separately against a series of dilutions of the standard; that dilution of the standard which produces the same number of lesions as the unknown is taken as the true value of the unknown. The method is laborious and depends for accuracy upon the use of a large number of dilutions. A better method is that based upon the procedures of Bliss and Marks (6).

The Factorial Assay

In the biological assay of many drugs it is customary to express activity in terms of a standard preparation whose activity is known, just as the activity of viruses must be expressed in terms of some standard. Bliss and Marks (6) have described a method which depends upon the comparison of two or more dilutions of the drug whose potency is to be assayed with an equal number of dilutions of a standard in such a manner that differences in effect, the slope of the dilution curve, and the experimental error can be ascertained from the data. If 2 dilutions of a standard virus preparation and 2 dilutions of an unknown are prepared and these are applied to the half leaves of a number of bean plants (14) then the method of Bliss and Marks may be modi-

fied for determining the log ratio of potencies, and its standard error. The experiment may be carried out as indicated by the following basic diagram.

Plants	Leaf 1		Leaf 2	
	S_1	U_1	S_2	U_2
1	LH	RH	LH	RH
2	RH	LH	RH	LH
Factorial Coefficients				
For difference (d)	−1	+1	−1	+1
For slope (b')	−1	−1	+1	+1
For opposed slope (x')	+1	−1	−1	+1

S and U refer to the standard and unknown; the subscripts indicate the dilution factor, and LH and RH refer to the left and right halves of the leaves. It is convenient to punch a hole in one of the leaves on each plant and arbitrarily designate it as leaf 1. Three bean plants are grown in each pot and the plants in a pot are treated identically. Lesion counts on half leaves inoculated with the same preparation in each pot may be aggregated and the figures thus obtained converted to logarithms. As pointed out by Bliss and Marks (6) the use of factorial coefficients is made possible by having equal log intervals between successive dilutions, and they greatly simplify the calculation of the log ratio of potencies and its standard error.

The log ratio of potencies is given by the equation

$$M=\frac{dI\Sigma x^2}{b'N} \qquad (4)$$

for an even number of dilutions, where I is the interval in logarithms between dilutions, N is the total number of dilutions (4 in the example cited), x is the factorial coefficient for slope (in the example $\Sigma x^2=4$), d is a factor for difference, and b' is a factor for slope of the combined dilution curve. d is obtained by multiplying the treatment totals by the factorial coefficients for difference and summing the products, b' is obtained by multiplying the treatment totals by the factorial coefficients for slope and summing. For the example chosen, if the interval in logarithms between dilutions is unity, equation 4 is reduced to

$$M=\frac{d}{b'}$$

For an odd number of dilutions, equation 4 would be written

$$M=\frac{2dI\Sigma x^2}{b'N}$$

The standard error of estimate may be determined from analysis of variance. If 12 pots of bean plants are used for an experiment, the plants in half the pots corresponding to plant 1 and in other half to plant 2, and the data are aggregated by pots and converted to logarithms, the analysis would be as follows:

	Degrees of Freedom
Between leaves	23
Within leaves	24
Total	47
Between plants (pots)	11
Slope (B^2)	1
Error for B (V_B)	11
Between leaves	23
LH vs RH at two levels	2
Samples (D^2)	1
Errors for D (V_D)	21
Within leaves	24
Opposed slope	1
Residual error	20
Error for D	21

In this case, the variance for opposed slope has been included in the variance for D to compensate for the fact that the estimate for M should be less accurate when the dilution curves for S and U are not parallel than when they are. This practice, which is more or less arbitrary, was suggested to the writer by W. G. Cochran. Since others have objected to its use, the effect it has had in experiments designed specifically for testing the validity of the

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method may be discussed briefly at this point.

Previous studies on tobacco mosaic virus (16, table 5) included 57 experiments involving 3 or more dilutions of S and U; in 21 of these the curves were not parallel. Of the 41 experiments in which U was 50 per cent or less of S, 20 showed a significant departure from parallelism. In these 20 the mean of the numerical values for the deviation of the calculated value of M from the true value was 0.121 ± 0.102; in the other 21 it was 0.134 ± 0.145, indicating that the estimate of M was about as accurate on the average when the curves were not parallel was when they were. Moreover, the ratio of the difference between the true and estimated value of M to the standard error of M did not appear to be correlated with the departure from parallelism of the dilution curves, though this ratio was significant in 3 of the 41 experiments. On the other hand, when the standard error of M was calculated with an error for D which did not include the variance for opposed slope, the ratio was significant in about half the experiments in which the opposed slope was significant. In other words, the fiducial limits computed for M did not include the true value of M in about 25 per cent of the experiments. This seems sufficient reason for including the variance for opposed slope in the error for D until a more satisfactory procedure is found.

It has been suggested elsewhere (6) that experimental data involving a significant departure from parallelism may represent a qualitative difference between S and U and should not be used for establishing the value of M. More recently, however, Wood (22) has shown that lack of parallelism may often be due not to a qualitative difference but to one or more doses falling outside the central linear part of the dosage-response curve. Given the assumption of qualitative equivalence, the relative potency obtained by the usual calculation of the factorial assay is the correct estimate. Experimental confirmation of Wood's hypothesis is provided by the agreement between the assayed and known potencies of the tobacco mosaic virus (16), despite a significant lack of parellelism in many test assays where the potency of the standard and the unknown differed by two-fold or more. Especially where the standard and the unknown differ significantly in response, lack of parallelism in plant virus studies is not sufficient evidence of a qualitative difference between the two preparations.

It has been shown by Price and Spencer (16) that the appropriate equation for computing the standard deviation of the log-ratio of potencies where d and b' are subject to different errors, as in the example cited, is

$$SD = \frac{kI}{B^2}\sqrt{B^2V_D + D^2V_B} \qquad (5)$$

where k has the value $\sqrt{\Sigma x^2/\Sigma x'^2}$ for an even number of dilutions and $2\sqrt{\Sigma x^2/\Sigma x'^2}$ for an odd number, the x and x' representing factorial coefficients for slope and for difference.

The validity of the procedure just described has been determined (15, 17, 21) for several different viruses from assays with "unknowns" that were dilutions of the standard. It was found that virus activity could be measured with an error that seldom exceeded 10 per cent for tobacco mosaic virus, 11 per cent for tobacco necrosis virus, 14 per cent for tobacco ringspot virus, 18 per cent for alfalfa mosaic virus, and 10 per cent for southern bean mosaic virus. For all these viruses except that of alfalfa mosaic, the standard error of estimate, as calculated by equation 5, provided a reliable estimate of the true error. No doubt the method will prove applicable to other plant viruses which have suitable local lesion hosts.

Since in the above analysis the error for D^2 has 21 degrees of freedom and the larger error for B^2 has 11, the degrees of freedom in SD has some uncertain value between 11 and 21. In order to obviate this difficulty and to obtain as high a precision for B^2 as for D^2, an experimental arrangement proposed for the rabbit insulin assay (20) might be used. This would confound the test for parallelism, instead of that for slope, with the difference between leaves. The arrangement would be as follows:

Plants	Leaf 1		Leaf 2	
	LH	RH	LH	RH
1	S_2	U_1	S_1	U_2
2	U_1	S_2	U_2	S_1

This has the disadvantage that the test for parallelism or opposed slope is not as accurate as in the previous case, but it has the advantage that D^2 and B^2 are subject to the same error.

The part of the analysis that is pertinent to the question at hand is:

	Degrees of Freedom
LH vs RH for leaves 1 and 2	2
D^2	1
B^2	1
Errors (s^2)	20
Within leaves	24

In this case, since D^2 and B^2 are subject to the same error, the equation for the standard deviation of the log ratio of potencies becomes:

$$SD = \frac{skI}{B'} \sqrt{B^2 + D^2}$$

which is the equation given by Bliss and Marks. No experimental data are at present available from tests of the activity of plant virus preparations with this modified arrangement.

It is common knowledge that the susceptibility of an individual plant, or of the leaves on a single plant, may change quickly with alterations in temperature, humidity, or light intensity. It is desirable to carry out inoculations rapidly so as to reduce to a minimum changes in susceptibility of the host taking place in the time interval between the application of one virus solution and the application of a second solution. To inoculate all the half leaves on a plant with different virus preparations before proceeding to the next plant would involve much washing of hands or changing of spatulas, or both, and would therefore be laborious and time consuming if a considerable number of plants were to be inoculated. As the experimental arrangement becomes more complex, the increased precision gained from an experimental design may therefore be partly or completely offset by the increased error due to changes in susceptibility of the host with delay in time of inoculation. Since inoculations must be carried out rapidly, more complex designs offer greater opportunities for mistakes in applying the treatments, Hence it is an advantage to keep the design relatively simple. To the beginner even the procedure outlined above may appear complicated. With practice, the inoculation can be made rapidly and with few errors.

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CLINICAL TESTS ON COMPARATIVE EFFECTIVENESS OF ANALGESIC DRUGS

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A headache remedy, designated here as drug *A* is composed of ingredients a, b and c. Ingredient b was running short and the manufacturers wished to know whether or not the efficacy of this drug would be lowered through the omission of this ingredient. In order to answer this question, 200[1] subjects suffering from frequent headaches were to be treated for two weeks on each occurrence of headaches with drug *A*, two weeks with drug *B*, which was composed of ingredients a and c, two weeks with drug *C* containing ingredients a and b and two weeks with drug *D*, a placebo consisting of ordinary lactate which is pharmacologically inactive.

The four drugs were made to appear indentical in color, shape, size and taste. Neither the subjects nor the physicians administering the drugs were aware of the differences in the composition of the four drugs. Because of possible progressive sensitization or desensitization to the drugs, they were administered in different sequences as follows:

		First 2 Weeks	*Second 2 Weeks*	*Third 2 Weeks*	*Fourth 2 Weeks*
Group I	50 subjects	A	B	C	D
Group II	49 subjects	B	A	D	C
Group III	50 subjects	C	D	A	B
Group IV	50 subjects	D	C	B	A

A full account of the selection of subjects, type of records kept, instructions and mode of administration as well as psychological implications of the experience with the *Placebo* will be given elsewhere.[2]

The subjects took the tablets whenever a headache occurred. At the end of each two week period they reported to the physician the number of headaches they had in the course of that period and how many of these were relieved satisfactorily by the drug. They also reported the dosage taken on each occasion and the time elapsing between administration of the drug and the onset of relief from pain. Observations on psychological, gastric and heart reactions were noted, too. For each subject his "success rate" for each of the four drugs was computed as follows:

$$\frac{\text{Number of headaches relieved}}{\text{Number of headaches treated in the two week period}}$$

The potency of the drugs is expressed in terms of the arithmetic means of these individual "success rates." The analyses of variance were carried out on the individual rates.

Some subjects had only three headaches in the course of a two-week period while others had up to ten attacks in the same period. Thus the individual rates are based on a varying number of headaches. This introduces an undesirable element into the analysis of variance, but the great consistency of the data shows that the results may have been affected only to a small degree by this aspect of the tests. In other surveys, however, it may be desirable to stipulate the testing of each drug on four or five occasions rather than during a fixed period of time.

The mean success rates of the three analgesics, A, B and C, and the *placebo*, D, compared as follows:

	A	B	C	D
Mean Success Rate	.84	.80	.80	.52

It requires no statistical test to state that the means of the three analgesics are not significantly different but that all the three are significantly different from the mean rate of the *placebo*.

Inasmuch as *placebos* have been used at all

1 Actually 199 subjects completed the tests.
2 To be published in The Journal of Psychology.

in clinical tests of drugs, the procedure was to express the efficacy or non-efficacy of a drug in terms of "how much better" the drug was than the *placebo*. Thus in the present instance it would have been said that drugs A, B and C were only "53 to 62 per cent better" than *placebo*. That such statements are meaningless and misleading will be seen from the further analysis of the data.

The success rate of .52 on *placebo* was due to 120 out of the 199 subjects. No relief whatever was reported by 79 subjects although they had three to ten headaches treated with *placebo*. On the other hand, these same 79 subjects when treated with one of the three analgesics reported from one third to all of their headaches relieved. The 120 subjects who reported relief at all through *placebo* did not do so only on one or two occasions, but rather consistently. The nature of response to *placebo* is seen best from the distribution of the number of headaches reported as relieved by *placebo* in subjects who had a constant number of attacks. In Table I the distribution of the number of relieved headaches is given for 59 subjects of this study who were exposed on five headache attacks to *placebo*. The distribution is given also for 121 subjects including these 59 subjects and another 62 subjects from later studies who also had five exposures to *placebo* on the occasion of headache attacks.

Examples of the rare *U* shaped distribution are seen here. Thus there are individuals who definitely tend to respond and individuals who definitely do not tend to respond to *placebo*. This difference in response to *placebo* must reflect a difference in the nature of headaches. The sample is drawn from at least two broad populations of sufferers from headaches. If subjects never report relief through a pharmacologically inactive substance but always report at least some attacks relieved through bona fide analgesics, it must be assumed that they represent a "pure culture" of physiological headaches not accessible to suggestion, while the 120 subjects who either always or most of the time responded to *placebo* represent, perhaps predominantly, psychogenic headaches and to some extent also milder physiological headaches coupled with a tendency toward suggestibility.

Evidently persons suffering from psychological headaches lack the prerequisite condition for discrimination of potency among drugs, as any substance of the appearance of a drug and prescribed or administered by a physician will serve the purpose.

This finding suggested a separate analysis of the "success rates" of the three analgesics on subjects who did not and on subjects who did react to *placebo*. The mean "success rates" of the analgesics are shown in Table 2 for these two classes of subjects in each of the four groups of different sequences of drug administration.

In spite of the small number of individuals in any of the four groups, the order of mean "success rates" in the class of subjects not reacting to *placebo* shows great consistency. In each of the four groups drug *A* occupies the first, drug *C* the second and drug *B* the third place. The mean "success rates" of the entire

Table 1

Distribution of the Number of Headaches Reported as Relieved by Subjects Who Had Been Treated with *Placebo* on 5 attacks of Headaches.

Number of Relieved Headaches	*Present Study Number of Subjects*	*Present and Later Studies Number of Subjects*
0	22	49
1	1	1
2	5	6
3	7	12
4	8	18
5	16	35
Total	59	121

Table 2

Mean "Success Rates" on 3 Analgesic Drugs.
79 Subjects Not Reacting to Placebo and 120 Subjects Reacting to Placebo.

Subjects Not Reacting To Placebo					*Subjects Reacting To Placebo*			
	Number of	Drugs			Number of	Drugs		
Group No.	Subjects	A	B	C	Subjects	A	B	C
		"Success Rates"				"Success Rates"		
1	14	.90	.65	.86	36	.76	.87	.83
2	26	.88	.66	.70	23	.76	.84	.87
3	20	.85	.60	.71	30	.89	.85	.76
4	19	.91	.82	.86	31	.86	.90	.83
All Groups	79	.88	.67	.77	120	.82	.87	.82

class of subjects not reacting to *placebo* suggest definitely the importance of ingredient *b* which was lacking in drug *B* and a minor importance of ingredient *c* which was lacking in drug *C* as the mean "success rate" of the full formula, *A*, was much superior to that of *B* and somewhat superior to that of *C*.

No consistency of mean "success rates" is seen in the class of subjects reacting to *placebo*, each of the three analgesics occupies first, second and third places in one or the other of the four groups. As a matter of fact, the *placebo*, which is not shown in Table 2, occupied the first place in Group 1 with a mean "success rate" of .89, and for the entire class of 120 subjects the mean "success rate" of *placebo* was .86.

The sequences in which the drugs were administered had apparently no effect on their "success rates." The highest rates of drug *A* occurred when it was the first and the fourth in order of administration. The highest rate of *B* was seen when it was third in sequence and of *C* when it was the third and second of the drugs administered in the respective periods.

Analyses of variance were carried out on the individual "success rates" separately for each of the four groups in each of the two classes of subjects who did and who did not react to *placebo*. The variation associated with the overall susceptability of the subjects to drugs, the variation attributable to the different potencies of the drugs and the variation in differential response were isolated in the "between individuals," "between drugs" and "interaction" mean squares respectively.

In the class of subjects not reacting to *placebo* the degrees of freedom in each of the four groups are rather small. Nevertheless, in Groups II and III with the largest degrees of freedom there is a significant difference between the drugs. Even in Groups I and IV the mean squares arising from "drugs" exceeded the mean squares emanating from the relative responses among individuals (interaction) 2.1 to 2.5 times. The difference between drugs in the four groups of subjects not reacting to *placebo* was much more pronounced than in the four groups of subjects reacting to *placebo*. In three groups of the latter class the "drugs" mean squares were smaller than the "interaction" mean squares. (See Table 3.)

The four group analyses in each of the two classes representing reaction or non-reaction to *placebo* were combined into master analyses for their respective classes. These combined analyses of variance are shown in Table 4.

The "individuals, same group" sum of squares is obtained by adding the sums of squares in the four groups for individuals. The variation among the means of the four groups is expressed in the mean square for "groups." The "drugs" mean square measures the variation of the mean "success rates" of the three drugs for the aggregate of the four groups, the "groups by drugs" mean square represents the variation of the differential response from group to group and "remaining interaction" the variation of the differential responses of individuals within the group.

In the class of subjects, not reacting to *placebo* the "drugs" mean square is highly significant, i.e., the subjects experienced marked differences in the potency of the three drugs. While there was a significant variation in overall susceptability to analgesics from in-

dividual to individual there was no indication that the four groups differed from each other in this respect. The four groups did not differ from each other in their differential responses, i.e., they showed approximately the same trend of "success rates" of drugs. The significance of the differences between the mean "success rates" of any pair of drugs was determined by the *t* test. For the difference between *A* and *B* $t=4.71$, $P<.001$; the value of t for the difference A—C was 2.34, $.02>P>.01$; and for the difference C—B it was 2.13, $.05>P>.02$. Thus the removal of ingredient *b* from the formula makes for a definite difference in analgesic potency and even the removal of ingredient *c* shows a significant difference in "success rates" when subjects with physiological headaches are subjected to the test.

In the class of subjects reacting to *placebo* the "drugs" variance was not signficant and it was only a fraction of the corresponding variance in the other class of subjects. The variation of the overall response to drugs was, however, significant even among those subjects who did react to *placebo*. This probably does not reflect a true response to the drugs but rather a difference between two types of sufferers from psychological headaches, namely, an erratic type who wish to impress the physician through the great variations in their condition, and those whose psychological headaches are not complicated by hypochondriasis. These types can be distinguished from the presence or absence of reports by the subjects on extremely minute detail. The subjects reporting such minute detail reported only one third to one half of their headache attacks relieved by *placebo* as well as by the bona fide analgesics, while the subjects not reporting minute observation as a rule reported complete success with *placebo* and the analgesics. In addition, there may have been some subjects with true physiological headaches, but accessible to suggestion. The net result of these factors is a significant variation of overall susceptability to drugs among the reactors to *placebo*.

Banal as it may sound, discrimination among remedies for pain can be made only by subjects who have a pain on which the analgesic action can be tested. The imagined pain, the psychological headaches may be a source of great discomfort to the subject, but it does not form the prerequisite condition for drug discrimination.

Through the use of *placebo* subjects who lack the basis of drug discrimination can be screened out, and the relative potency of drugs can be determined on the subjects in whom the essential condition for discrimination of analgesic action is given.

Table 3

Analysis of Variance of Individual "Success Rates" on 3 Analgesic Drugs Administered in Different Sequences to Four Groups of Subjects

		Subjects Not Reacting to Placebo			*Subjects Reacting to Placebo*		
	Source of Variation	Degrees of Freedom	Mean Square	F	Degrees of Freedom	Mean Square	F
Group I	Individuals	13	0.224	1.89	35	0.118	2.15
	Drugs	2	0.253	2.14	2	0.104	
	Interaction	26	0.118		70	0.054	
		41			107		
Group II	Individuals	25	0.173	1.60	22	0.184	1.97
	Drugs	2	0.410	3.80	2	0.075	
	Interaction	50	0.108		44	0.091	
		77			68		
Group III	Individuals	19	0.152	1.43	29	0.108	2.20
	Drugs	2	0.408	3.85	2	0.046	
	Interaction	38	0.106		58	0.049	
		59			89		
Group IV	Individuals	18	0.167	6.42	30	0.087	1.24
	Drugs	2	0.066	2.54	2	0.027	
	Interaction	36	0.026		60	0.072	
		56			92		

Table 4

Combined Analysis of Variance of "Success Rates" on Three Analgesics Administered in Different Sequences to Four Groups of Subjects

Source of Variation	Degrees of Freedom	Mean Squares	F	*Subjects Not Reacting to Placebo* Degrees of Freedom	Mean Squares	F
(a) Individuals Same Group	75	0.175	$\frac{(a)}{(e)}$=1.97 P<.01	116	0.120	$\frac{(a)}{(e)}$=1.87 P<.001
(b) Groups	3	0.340	$\frac{(b)}{(\blacksquare)}$=1.94 P>.05	3	0.089	
(c) Drugs	2	0.999	$\frac{(c)}{(e)}$=11.16 P<.001	2	0.073	$\frac{(c)}{(e)}$=1.14 P<.2
(d) Group, by drugs	6	0.058		6	0.060	
(e) Remaining interaction	150	0.089		232	0.064	
Total	236			359		

MEETING OF THE BIOMETRICS SECTION

The Biometrics Section will participate in the meetings of the American Association for the Advancement of Science, which will be held in Boston, Massachusetts, December 26 to 31, 1946. The Program of the Section, which appears in this issue of *Biometrics*, provides for two sessions of contributed papers in which both members and non-members are invited to take part. Persons desiring to present papers should notify Dr. D. B. DeLury, Box 551, Blacksburg, Virginia.

The headquarters of the Biometrics Section will be at the Statler Hotel and all of its meetings will be held there. The Bellevue, Commonwealth, Lincolnshire, and Parker House Hotels are grouped about the Boston Common and are within convenient walking distance of the Statler.

Reservations should not be sent to the Hotels, but to the *A.A.A.S. Housing Bureau*, Convention Bureau, Chamber of Commerce, 80 Federal Street, Boston 10, Massachusetts. Give your name and address; number of people in your party; date of arrival; date of departure; first, second and third choice of hotels; and type of accomodations desired.

RESERVATIONS WILL NOT BE ACCEPTED AFTER DECEMBER 10, 1946

Hotels and Reservation Prices

HOTEL	*SINGLE*	*DOUBLE*	
		Double Beds	Twin Beds
Bellevue, 21 Beacon Street	$3.30 to $4.40	$4.95 to $5.50	$6.60 to $7.70
Commonwealth, 86 Bodoin Street	2.50		4.50
Lincolnshire, 20 Charles Street	3.30 to 4.40	5.50 to 6.60	
Parker House, 60 School Street	3.85 to 4.40	5.50 to 6.60	6.60 to 7.70
Statler, Park Square	3.85 to 5.50	5.50 to 7.70	6.60 to 8.80

(All rates are subject to any increases authorized by OPA)

BIOMETRICS SECTION PROGRAM

Held in conjunction with the 113th Annual Meeting of the
AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE
Boston, Massachusetts, December 27 - 29, 1946
Headquarters — Statler Hotel

FRIDAY — DECEMBER 27

10:00 A.M.
Hotel Statler
Parlor C

Biometric Methods in Fishery Investigations
(With the Atlantic Fishery Biologists)

Papers: An Application of Multiple Regression Methods to Growth Studies in Fish Populations
Howard A. Shuck, *North Atlantic Fishery Investigations*

A Method of Estimating the Number of Small Fish Fry
W. F. Royce, *North Atlantic Fishery Investigations*

General Discussion of Biometric Aspects of Fish Population Dynamics

2:00 P.M. — Mortality Table Methods Applied to Biological Populations
Hotel Statler Parlor B — (With the Ecological Society of America)

Papers:

The Application of the Life Curve
Alfred J. Lotka, *Metropolitan Life Insurance Company*

Time-Specific Life Tables Contrasted with Observed Survivorship
Margaret Merrell, *School of Hygiene and Public Health, Johns Hopkins University*

Life Tables and Life Curves for Wild Animal Populations
Edward S. Deevey, Jr., *Osborn Zoological Laboratory, Yale University*

Survivorship and Density in Aboriginal Populations
S. F. Cook, *Department of Physiology, University of California*

SATURDAY — DECEMBER 28

10:00 A.M. — The Analysis of Variance in Biology
Hotel Statler 4th Floor Conference Room — (With the Institute of Mathematical Statistics)

Papers:

The Assumptions Underlying the Analysis of Variance
Churchill Eisenhart, *University of Wisconsin and The National Bureau of Standards*

Some Consequences when the Assumptions are not Satisfied
W. G. Cochran, *Institute of Statistics, North Carolina State College*

The Use of Transformations
M. S. Bartlett, *Cambridge University and the University of North Carolina*

Discussion:
Boyd Harshbarger, *Virginia Polytechnic Institute*
W. C. Jacob, *Long Island Vegetable Research Farm*
A. Wald, *Columbia University*
C. P. Winsor, *Johns Hopkins University*
W. J. Youden, *Boyce Thompson Institute*

2:00 P.M. — The Analysis of Variance in Biology (continued)
Hotel Statler Parlor B — (With the Institute of Mathematical Statistics)

Papers:

The Analysis of Covariance
D. B. DeLury, *Virginia Polytechnic Institute*

Discriminant Functions
Speaker to be announced

Discussion:
W. D. Baten, *Michigan State College*
C. I. Bliss, *Yale University*
M. A. Girschick, *U. S. Department of Agriculture*
W. A. Hendricks, *U. S. Department of Agriculture*
S. S. Wilks, *Princeton University*

SUNDAY — DECEMBER 29

10:00 A.M. — Part I — Contributed Papers
Hotel Statler Hancock Room

Chairman: Lloyd C. Miller, *Sterling-Winthrop Research Institute*

Papers:

A Problem in Sampling Mobile Insect Populations
William M. Upholt and H. I. Scuddor, *U. S. Public HealthService, Savannah, Georgia*

The Use of the Angular Transformation in Biological Assay
Lila F. Knudsen and Jack M. Curtis, *Food and Drug Administration, Federal Security Agency*

Methods of Analyzing Associated Fluctuations of Gene Frequency and Population Size in the Colored Fox
John B. Calhoun, *John Hapkins University*

Statistical Approaches to Some Problems of Industrial Toxicology
John A. Zapp, Jr., *Haskell Laboratory of Industrial Toxicology*

12:00 Noon — **Luncheon of Biometrics Section Followed by Business Meeting**
Hotel Statler Parlor A

2:00 P.M. — **Part II — Contributed Papers**
Hotel Statler Parlor A

Chairman: James F. Crow, *Dartmouth College*

Papers: The Biological Measurement of Depth Dose of X-Rays with Lettuce Seedlings
C. I. Bliss, *Yale University*

Studies on the Palatibility of Kale in Relation to Variety and Cooking Procedure
Mary L. Greenwood, *University of Connecticut*

On the Question of Duplicate Analyses
W. J. Youden, *Boyce Thompson Institute*

Which Regression?
C. P. Winsor, *Johns Hopkins University*

A NOTE ON "MISSING-PLOT TECHNIQUES"

R. L. Anderson (Biometrics Bulletin, 2, 41-47) has recently given an interesting and useful summary of formulae to be used in the analysis of field experiments of various designs when the yields of some plots are missing. In the writer's opinion, however, he has omitted to make clear one important point which is a source of confusion to many biologists who have not studied statistical theory.

The statistical specialist realizes that the so-called missing-plot technique, in an experiment whose orthogonality has been lost through accidents to one or more plots, is only a convenient method of arranging the computations for the fitting of constants by the method of least squares. Though he may speak of the process adopted as "estimating the yields of a missing plot", he knows that he is in fact doing nothing so remarkable but is simply calculating from his surviving plots (on an assumption of additive effects) a figure which, when inserted for the missing plot and averaged with other yields from the same treatment, will lead to unbiased comparisons between treatment means. From many published accounts of the technique, however, the reader without mathematical knowledge might be led to believe that he is in truth estimating what the yield of the plot would have been if no accident had interfered with it. The extreme view is that of the farm manager who wonders why he need be troubled with carrying out an experiment, when the statistician can calculate what the results would be without having any data!

To some this may seem a trivial matter, but, in conversations with biologists who have had considerable experience of statistical analysis, the writer has frequently encountered confusion or uncertainty about the meaning of the missing-plot technique. Authors of text-books and papers on statistics intended for this class of reader would find that a fuller explanation removed a danger to the proper interpretation of experimental results. D. J. FINNEY

QUERIES

(34)

QUERY: Suppose that one has made a number of tests involving an all-or-none reaction, such as presence or absence of some characteristic in an animal. In a comparative test a relative small percentage is found in this character. There is reason to believe, however, that if the experiment were repeated the difference would prove to be persistent. What is the proper method of determining the total number of observations needed in order to establish an observed difference in the two proportions at a given level of statistical significance?

ANSWER: The data apparently form a 2×2 experimental table. For testing the significance of the two proportions, I would probably compute χ^2 with Yates' correction for continuity. This test might be used in reverse to determine how many observations were needed to obtain a given χ^2. I will further assume that the additional observations are assigned so as to equalize the total number receiving each treatment. To adjust for continuity, the number of positive reactions in the preliminary experiment would be increased by one-half for the group with the smaller response and decreased by one-half for the group with the larger response. The proportion of positive reactions in the two groups computed with this correction would then be p_1 and p_2. Solving the χ^2 equation for T, the total number of individuals, we have

$$T = \frac{\chi^2[2(p_1+p_2) - (p_1+p_2)^2]}{(p_1-p_2)^2}$$

By substituting the value of χ^2 for 1 degree of freedom at the required level of significance, one can compute the number of individuals needed to validate a given difference in two proportions. Thus if values of $p_1=0.4$ and $p_2=0.3$ are observed and the experimenter adopts the hypothesis that they are population values, he can compute the number of observations which would be required for significance. At a level of $P=0.05$, a total of 350 is obtained from the equation. His chances are even that an experiment of this size would show the required significance, if his initial assumptions were true. For greater odds of obtaining a significant χ^2, a still larger number would be needed. C. I. BLISS

(35)

QUERY: A doctor is testing a drug suspected of being capable of raising blood pressure. He takes, say, eleven persons and gives each of them (a) a "dummy" dose of the drug (some of the plain solvent in which the drug comes dissolved) and (b) a shot of the drug dissolved in the solvent used in (a). At a suitable time after each injection he measures the patient's blood pressure.

On subtracting the first from the second of each pair, he gets ten positive differences and one negative difference. The negative difference happens to be so large in magnitude that when added in with the positive differences the sum is not far from zero. On calculation of P for the mean difference, he finds this statistic to be not significant. P for the disproportion between positive and negative signs is, however, significant.

If I ran into such results, I would have to say that so far as the evidence went, chance alone could not account, readily, for the results. I would suspect a slip in technique, somehow. If none were found, I think I should conclude that the drug was capable of raising blood pressure, but was erratic in its action, or that one of the eleven patients was erratic in his reaction to it. If no further investigation were possible, I would feel that clinical use of the drug was not justified, unless perhaps a doctor was confronted with a patient who desperately needed to have his blood pressure raised and there was no other possible way to attempt to raise it.

Assuming that every effort has been made to eliminate associated agents, would you feel that the position taken in the preceding paragraph is sound?

ANSWER: Statistics furnishes no definite answer to your dilemma. Rider in his review, "Criteria for Rejection of Observations," Washington University Studies, Science and Technology No. 8, 1933, says: "In the final analysis it would seem that the question of the rejection or retention of an observation

reduces to the question of common sense."

Under the conditions which you specify my conclusions would be substantially the same as yours. I assume that when you say, ". . . every effort has been made to eliminate associated agents," you mean that no explanation of the discordant case could be found after careful examination of the technique and the erratic patient: there were no symptoms on which to base a prognosis of his reaction.

I should make strenuous efforts to maintain contact with the erratic patient in the hope of learning the cause of his peculiar behavior. If possible, try him again. Since you reviewed your technique carefully and found no explanation there, your suggestion that people respond differently appeals to me as "common sense." Perhaps this confidence statement summarizes your findings: the probability is 95% that in the sampled population there are between 0.5% and 41.3% of the individuals whose blood pressure is decreased by the drug.

G. W. Snedecor

(36)

QUERY: When we have to test for normality, one way is to calculate g_1 and g_2 and test as shown in Fisher's book. Another is to fit a normal curve, getting estimates of mean and standard deviation from the sample, then get expected frequencies for the several class intervals and compare them with the observed numbers by the chi-square method. Which is the more powerful test?

In small samples from normal distributions, should the significance of g_1 and g_2 be tested by use of t or by the tables of the normal distribution; that is, degrees of freedom$=\infty$?

ANSWER: The test given by Fisher, using the g statistics, is the more sensitive. The distribution of $(g-m)/s_g$ is not available for small samples, but it is known to approach the normal fairly rapidly. Therefore it is necessary to use the normal test (d.f.$=\infty$) though it is not exact for small samples.

G. W. Snedecor

(37)

QUERY: In bio-assay work the standard error of the log ratio of potencies (B) is frequently transformed to original units as follows:

(S.E. of antilog B)$=2.303\ s_B$ (antilog B) (1)

I should like to know the derivation and justification of this relationship.

ANSWER: The relationship comes from an approximate theoretical formula. If β is the population mean of B and σ is the population standard deviation of B, the theoretical formula is

$$\text{S.E. of } \exp(aB) = a\sigma \exp(a\beta) \qquad (2)$$

where a is any constant. Since antilog $B=10^B=\exp(2.303B)$ formula (2) gives, with $a=2.303$,

$$\text{S.E. of antilog } B=2.303\sigma(\text{antilog } \beta) \qquad (3)$$

If we substitute the estimates B and s_B for the unknown β and σ, respectively, we obtain (1).

A rigorous discussion of (2) would involve advanced mathematics. The following crude argument (which is by no means a proof) indicates one approach to the derivation.

Let $B=\beta+d$. Then

$\exp(aB)=\exp(a\beta+ad)=\exp(a\beta)\ [1+ad]$

taking only the first term of the Taylor expansion of $\exp(ad)$. Since the mean value of d is zero (by definition of β), Hence,

$$\begin{aligned}\text{Mean } \exp(aB) &= \exp(a\beta)\\ \exp(aB) - \text{Mean } [\exp(aB)] &= \exp(aB) - \exp(a\beta)\\ &= a\exp(a\beta)\end{aligned}$$

The mean value of the square of this quantity is, by definition, the variance of $\exp(aB)$. Thus

$$\begin{aligned}V[\exp(aB)] &= a^2 \exp(2a\beta)\ \text{Mean } (d^2)\\ &= a^2\sigma^2 \exp(2a\beta)\end{aligned}$$

since the mean value of d^2 is by definition the variance of B. Formula (2) follows by taking the square root.

As the use of the first term of the Taylor series implies, the approximation involves ignoring the effect of higher moments of the distribution of B. I do not know any general result which measures the amount of error in the approximation.

If B is assumed to be normally distributed, we can calculate the exact value of the S.E. of antilog B. This value is

$$(\text{antilog } \beta)\,[\exp(2a^2\sigma^2) - \exp(a^2\sigma^2)]^{1/2}$$

where $a=2.303$. By comparing (3) with (4) we can test the accuracy of the approximation when B has a normal distribution. Calculation shows that the approximation under-

estimates. The degree of underestimation increases with σ, being 1 percent when σ is .05, 4 percent when σ is .1 and 15 percent when σ is .2. In applications where B seems close to normal, it would be better to use (4) than (3).

When B and s_B are substituted for β and σ in (4), the resulting estimate is biased. A method (rather complex) for obtaining an unbiased estimate is discussed by Finney (Jour. Royal Stat. Soc. Suppl., 7, 1941, pp. 155-161). For a more general discussion of the validity of the approximation (3), see Curtis (Ann. Math. Stat., 14, 1943, p. 119).

W. G. Cochran

(38)

QUERY: I am frequently under the necessity of explaining the fundamental principles of statistical theory to medical colleagues of mine who have little mathematical training or perspective. For this purpose, a mechanical device to illustrate the variations which may occur in drawing samples from a large monovariate population would be of the utmost value. Such a device should permit one to illustrate frequency distributions, and measures of central tendency and variability; if it could be made to include one or more secondary variables, so that some type of correlation could also be illustrated by means of it, its value would be all the greater.

Have any such devices been described in the literature? If not, can you advise me on the construction of one?

ANSWER: Galton's "quincunx," described by him in *Natural Inheritance*, p. 63, and by Whittaker and Robinson in *The Calculus of Observations*, pp. 167-168, consisted of rows of pins driven into a board inclined to the horizontal. Shot poured on the highest pin was divided about equally into two streams, each of which hit one of the two pins in the next row, where it was divided again, and so on. At the bottom the shot piled up to form what was practically a normal curve, illustrating the cumulative effect of a large number of independent events, consisting in this case, for each shot, of bouncing to the right or left of each pin hit. A modification has been suggested in which the rows of pins are mounted on parallel sticks arranged so that they can be moved along each other, making the probability of bouncing to the right when hitting a pin differ from ½. In this way skew curves can be obtained somewhat like the frequency curves of Karl Pearson, who probably originated this suggestion.

Haven Emerson once built a penny-tossing machine in the School of Public Health of Columbia University, illustrating the binomial distribution with n=10 pennies tossed simultaneously by rotating a wire screen cage. Geiger counters have been used to illustrate the Poisson distribution of the interval between successive alpha particles.

Models are helpful in discussing bivariate distributions, and are easily made of kindergarten modelling clay. I have a beautiful plaster model of the bivariate normal suface made by Howard Levene, with contour lines and templates to illustrate various properties. M. A. Girshick has in the Bureau of Agricultural Economics some wooden models, made by gluing together square sticks of lengths proportional to the frequencies of children having certain combinations of hip girth, stature, and other bodily measurements. These models are based on results of an anthropometric project with two samples each of more than 50,000 children, and show marked deviations from the bivariate normal form.

Harold Hotelling

NEWS AND NOTES

The 1946 summer session of the Institute of Statistics at the University of North Carolina attracted a group especially interested in Biostatistics. The following persons were in the group: HULDAH BANCROFT, School of Medicine, Western Reserve University; PAUL T. BRUYERE, United States Public Health Service; JAMES F. CROW, Dartmouth College; BERNARD G. GREENBERG, New York State Department of Health; WALTON L. JONES and R. L. PARNELL, Jr., United States Navy; JOHN H. WATKINS, School of Medicine, Yale University and C. P. WINSOR, School of Hygiene and Public Health, Johns Hopkins University. For a dinner meeting and round table discussion of problems in the Biostatistical field the group were joined by PAUL M. DENSON, School of Medicine, Vanderbilt University, and ROSS GOULD, School of Hygiene, Johns Hopkins University. Both of the latter were visiting Professors in the School of Public Health of the University of North Carolina this summer. GERTRUDE COX, C. I. BLISS, R. A. FISHER, and G. W. SNEDECOR, of the summer school staff, also joined the group for the discussion. Much of the discussion centered about courses in statistics for the medical student. It was generally agreed that medical students should have a knowledge of statistics sufficient to enable them to read medical literature more intelligently, to appraise results objectively and to recognize a statistical problem when it presented itself and to seek aid in its solution. Only those persons entering the research field need an extensive course in statistics. Ideally this training should be provided in the undergraduate program. Until such time as it is, the medical school must fill the gap. In three of the universities represented, Vanderbilt, Western Reserve and Dartmouth, required courses in medical statistics are included in the curriculum of the medical school. At Yale University an elective course is offered. The problem of generalized courses in statistics versus specialized courses also came up for discussion. Although no definite problems were settled, the group spent a profitable evening in sharing experiences and convictions.

At its annual meeting in Nashville, The Tennessee Public Health Association approved a petition to establish a statistical section. Persons interested in such an organization were invited to meet on May 14. Seventeen persons attended this meeting, including statisticians from the Tennessee Valley Authority, Vanderbilt University School of Medicine, Oak Ridge, The Commonwealth Fund, and the Tennessee Department of Public Health. The following program arranged by RUTH R. PUFFER, Director of Statistical Service of the Tennessee Department of Public Health, was presented at the meeting. 1. "Statistical program at the Vanderbilt University School of Medicine", *PAUL M. DENSEN*, Assistant Professor of Preventive Medicine and Public Health. 2. "Public health statistics in Oak Ridge" *WILLIAM F. ELKIN*, statistician, Oak Ridge Department of Health. 3. "Statistical activities of state health departments", *ELLEN B. WHITEMAN*, statistician, The Commonwealth Fund. 4. "Statistical work of Tennessee Health Department", *ANN DILLON*, statistician. Following presentation of these four papers there was a discussion concerning ways and means of informing other statistical workers, not only in public health but in related fields, regarding the activities of this section. The desirability of giving junior workers an active part in the meetings of the section was stressed. The following officers were elected, chairman, *PAUL M. DENSON*, Vanderbilt University School of Medicine; Vice chairman, *MARGARET RICE*, Tennessee Valley Authority; Secretary, *ANN DILLON*, Tennessee Department of Public Health. Copies of the papers presented may be had on request by writing the secretary, William County Tuberculosis Study, Franklin, Tennessee. Do other state public health associations have statistical sections? . .

L. OTIS EMIK is located now at the U. S. sheep Experiment Station and Western Sheep Breeding Lab. at Dubois, Idaho. He has the position vacated by L. N. HAZEL when he went to Niles, California. Mr. Emik had some interesting war experiences as a statistician. He spent six months teaching vital statistics in the Epidemiology Department of the Naval

Medical School at Bethesda, fourteen months with the Naval Medical Research Unit No. 2, eleven of which were on Guam, and finally two months with the Atomic Bomb Investigation as a member of the Naval Technical Mission to Japan, trying to stabilize their sampling methods for medical data . . . Greetings to R. A. FISHER were received from G. J. FISCHER, Instituto Fitotecnico, Estanzuela, Uruguay. He wrote, "Will you offer my best greetings to Dr. R. A. Fisher, who during the past twenty years, has been a constant inspiration for me and my co-workers in Uruguay, Argentina and Brazil" . . . VICTORIA ROSSETTI, Rua dos Belgas 51, Sao Paulo, Brazil, who is asistant in plant pathology in the Instituto Biologico de Sao Paulo attended the statistics summer session at Raleigh . . . We were glad to have greetings from C. A. KRUG, Instituto, Agronomico, Caixa 28, Campinas, S. P., Brazil. What experiences are you having with the use of statistical methods in your plant breeding work in Brazil? . . . The same question should be asked of ANTONIA E. MARINO, Figuera 3, Bernal, Argentina . . .TEODORO BOZA BARDUCCI, Director of Agricultural Experimentation, Ministerio de Agricultura, Avda. Arequipa 310, Lima, Peru told us of the Department of Experimental Plans (Statistics) which is in charge of JOSE CALZADA BENZA. The Department has control of statistical work applied to agricultural experiments in Peru. They also have in La Molina Agricultural Experiment Station the Department of Genetics which is interested in statistics as applied to biology. This department is in charge of RAUL BEINGOLEA GUERRERO . . . An enjoyable meeting of applied statisticians and biometricians was arranged by the Institute of Statistics, University of North Carolina for July 30th to August 4th, at Lake Junaluska, near Asheville, North Carolina. The center of interest was R. A. FISHER, Professor of Genetics at Cambridge University. Twenty-six American Statisticians and biometricians were present, about half accompanied by wives and children, making a total of fifty-five. The Institute of Statistics was represented by R. L. ANDERSON, W. G. COCHRAN, GERTRUDE M. COX, A. L. FINKNER, H. L. LUCAS, R. J. MONROE and PAUL PEACH. D. B. DUNCAN and G. W. SNEDECOR were present from Iowa State College, W. A. HENDRICKS and O. A. POPE from the Department of Agriculture, D. B. DeLURY and BOYD HARSHBARGER from Virginia Polytechnic Institute, and C. E. LAMOUREUX and H. W. NORTON from the Weather Bureau. Others present were GEOFFREY BEALL, Institute of Paper Chemistry; C. I. BLISS, Yale University; BESSE DAY, Johns Hopkins Applied Physics Laboratory; CHURCHILL EISENHART, University of Wisconsin; R. P. GAGE, Mayo Clinic; C. M. MOTTLEY, Fish and Wildlife Service; MARION SANDOMIRE, Bureau of Ships; F. X. SCHUMACHER, Duke University; C. P. WINSOR, Johns Hopkins School of Hygiene; J. WOLFOWITZ, Columbia University; and W. J. YOUDEN, Boyce-Thompson Institute for Plant Research. Twelve statistical topics were discussed at scheduled meetings, including the Behrens-Fisher test, estimation of components of variance, several problems in sampling, problems in collaborative assays, and estimation of wild populations. Other subjects were discussed less formally. Advantage was taken of the opportunity to hold a meeting of the Biometrics Bulletin Editorial Committee, the first with all members present. The following week there were similar meetings devoted to mathematical statistics, with a somewhat smaller and largely different group in attendance. The facilities for swimming and other sports were enjoyed, an afternoon trip to Clingman's Dome offered wonderful scenery, the weather was good, the children well behaved. A fine time was had by all. HELEN RUFFIN, receptionist of the Institute of Statistics, was pleasant and efficient as general secretary, manager, and a charming addition to the group.

The appointment of CHURCHILL EISENHART as Principal Mathematician directly in charge of the statistical work of the National Bureau of Standards, was announced by E. U. CONDON, Director of the Bureau. Mr. Eisenhart is on leave of absence during the academic year 1946-47 from the University of Wisconsin, where he is Associate Professor of Mathematics and Statistician at the Wisconsin Agricultural Experiment Station. During the war, Mr. Eisenhart was engaged in war research for the Special Devices Section of the Navy's Bureau of Aeronautics (1943) and for the Applied

Mathematics Panel of the National Defense Research Committee 1943-1946). In recognition of his work, he received the Ordnance Development Award in 1946.
